

iGEM 2016: Pittsburgh
Week 1 Lab Notebook

Monday, May 23

Meeting, lab tour

Tuesday, May 24

LB broth (25 g/L)

[Poured plates](#) -- some didn't solidify even after time in cold room the next day

Catalogued

Wednesday, May 25

Streaked 1 plate competent cells (Top10, Alex), incubated overnight to grow

[Prepared solutions](#) for transformation (200 ml 50% glycerol, LB broth (2x 250 mL), .1 M CaCl₂, 1 M CaCl₂)

Bloodborne Pathogens training

Thursday, May 26

Remove cells from incubator; white streaks (morning)

4x liquid cultures into shaker/incubate, speed 225 (afternoon)

Friday, May 27

Starter culture into 500 mL LB broth, shake

0.1 CaCl₂ and 15% glycerol: 10 mL 1M CaCl₂, 30 mL 50% glycerol, 60 mL DI water

Into cold room

Density of cells = .49 and .51 into 3 centrifuge bottles. Ice for 20 min.

Centrifuge 3000g, 10 min

Pour off supernatant, resuspend .1 M CaCl₂, ice 30 min.

Finish [competent cell protocol](#)